

General Regression Neural Network and Monte Carlo Simulation Model for Survival and Growth of *Salmonella* on Raw Chicken Skin as a Function of Serotype, Temperature, and Time for Use in Risk Assessment[†]

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ABSTRACT

A general regression neural network (GRNN) and Monte Carlo simulation model for predicting survival and growth of *Salmonella* on raw chicken skin as a function of serotype (Typhimurium, Kentucky, and Hadar), temperature (5 to 50°C), and time (0 to 8 h) was developed. Poultry isolates of *Salmonella* with natural resistance to antibiotics were used to investigate and model survival and growth from a low initial dose (<1 log) on raw chicken skin. Computer spreadsheet and spreadsheet add-in programs were used to develop and simulate a GRNN model. Model performance was evaluated by determining the percentage of residuals in an acceptable prediction zone from −1 log (fail-safe) to 0.5 log (fail-dangerous). The GRNN model had an acceptable prediction rate of 92% for dependent data ($n = 464$) and 89% for independent data ($n = 116$), which exceeded the performance criterion for model validation of 70% acceptable predictions. Relative contributions of independent variables were 16.8% for serotype, 48.3% for temperature, and 34.9% for time. Differences among serotypes were observed, with Kentucky exhibiting less growth than Typhimurium and Hadar, which had similar growth levels. Temperature abuse scenarios were simulated to demonstrate how the model can be integrated with risk assessment, and the most common output distribution obtained was Pearson5. This study demonstrated that it is important to include serotype as an independent variable in predictive models for *Salmonella*. Had a cocktail of serotypes Typhimurium, Kentucky, and Hadar been used for model development, the GRNN model would have provided overly fail-safe predictions of *Salmonella* growth on raw chicken skin contaminated with serotype Kentucky. Thus, by developing the GRNN model with individual strains and then modeling growth as a function of serotype prevalence, more accurate predictions were obtained.

Salmonella is a leading cause of gastroenteritis and is often isolated from poultry foods, such as eggs, chicken, and turkey (1). There are more than 2,300 serotypes of *Salmonella*, yet only about 50 are responsible for most cases of gastroenteritis. One of the most important serotypes is Typhimurium, which causes 20% of human infections but 50% of human deaths (6). The top three serotypes isolated from chickens are Enteritidis, Kentucky, and Typhimurium, and those most frequently isolated from turkeys are Hadar, Senftenberg, and Heidelberg (6).

Parveen et al. (31) reported that the two most prevalent serotypes isolated from chickens prechill and postchill in a processing plant were Kentucky and Typhimurium and that over 80% of these isolates were resistant to antibiotics. Although antibiotic resistance is not desirable from a public health point of view, it is beneficial from a predictive microbiology perspective because it allows modeling of

Salmonella growth and survival on chicken products with native flora (25–27).

Variation of growth among serotypes of *Salmonella* has been reported (5, 16). On cooked chicken incubated at 25°C, the growth rate of Typhimurium is faster than that of Enteritidis but similar to those of nine other serotypes (17). Also, on cooked chicken incubated at 10 to 48°C, the optimal growth rate of Typhimurium is similar to that of Dublin but higher than that of Enteritidis (19). Whether growth of Kentucky differs from growth of other serotypes of *Salmonella* has not been reported.

Performance of predictive models can be improved by using better-fitting models. It has been reported that general regression neural network (GRNN) models outperform regression models and other types of neural network models in predictive microbiology applications (13, 30). With the advent of commercial software applications that perform GRNN modeling, it is now easy to use GRNN modeling in predictive microbiology studies. Moreover, GRNN modeling software is compatible with Monte Carlo simulation software. Thus, it is possible to create GRNN models that use Monte Carlo simulation to model uncertainty and variability of independent variables. This has been accom-

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plished for regression models (18, 21, 22) but not for GRNN models. Output distributions from such models can be used in risk assessment (20).

The objective of the present study was to develop a GRNN model that uses Monte Carlo simulation to provide stochastic predictions of survival and growth of *Salmonella* from a low initial dose on raw chicken skin with native flora as a function of serotype (Typhimurium, Kentucky, and Hadar), temperature (5 to 50°C), and time (0 to 8 h) for use in risk assessment. The temperatures investigated were selected to encompass the temperature range for growth of *Salmonella*, whereas the incubation times investigated were selected to encompass the times of temperature abuse encountered during poultry processing and during meal preparation.

MATERIALS AND METHODS

Salmonella serotypes. Isolates of serotypes Typhimurium, Kentucky, and Hadar were obtained from a poultry company. Typhimurium was resistant to chloramphenicol (C), ampicillin (A), tetracycline (T), and streptomycin (S). Kentucky was resistant to novobiocin (N), A, T, and S. Hadar was resistant to T, sulfasoxazole (U), gentamicin (G), and S. Stock cultures were maintained at -70°C in brain heart infusion broth (Difco, Becton Dickinson, Sparks, MD) that contained 15% glycerol (Sigma, St. Louis, MO). A multiple-antibiotic-resistant isolate of serotype Enteritidis was not available for inclusion in this study.

Plating media. Xylose lysine Tergitol 4 base agar medium without tergitol (XL) (Difco, Becton Dickinson) but supplemented with 25 mM HEPES (H) and 25 µg of C, A, T, S, N, U, or G (Sigma) per ml was used for plating.

Experimental design. A full $3 \times 10 \times 5 \times 2 \times 2$ factorial arrangement of serotype (Typhimurium, Kentucky, and Hadar), temperature (5, 10, 15, 20, 25, 30, 35, 40, 45, and 50°C), time (0, 2, 4, 6, and 8 h), trial (1 and 2), and sample (a and b) was used for model development. Replicate trials were conducted in separate weeks with different batches of raw chicken skin. Experimental errors resulted in loss of data from 20 of 600 samples.

Sample preparation. Raw chicken thighs were purchased weekly at retail stores. Skin was removed, placed on a cutting board, held at -20°C for 15 min, and cut into circular portions (2.14 cm²), which were placed on raw thigh meat in plastic jars with screw-cap lids and stored at 4°C for 24 or 48 h before use.

Sample inoculation. Five microliters of stock culture was added to 5 ml of BHI broth in a 25-ml Erlenmeyer flask that was sealed with a foam plug and incubated at 30°C and shaking at 150 rpm for 23 h. Skin portions were inoculated with 5 µl of a 10^{-7} dilution of the 23-h culture in buffered peptone water (Difco, Becton Dickinson). The initial logarithmic number was 0.95 ± 0.02 (mean \pm standard error of the mean) for Typhimurium, 0.78 ± 0.03 for Kentucky, and 0.91 ± 0.01 for Hadar.

Sample processing. Duplicate samples were processed per sampling time by placing a skin portion in a 207-ml plastic bag with filter screen (Whirl-Pak, Nasco, Fort Atkinson, WI) with 9 ml of buffered peptone water. Samples were pulsed (Pulsifier model PUL 100, Microbiology International, Frederick, MD) for 1 min, and the pulsifate was used for enumeration.

MPN. A three-tube most-probable-number (MPN) assay in buffered peptone water was used to enumerate *Salmonella* from 0 to 3.26 log per skin portion (25). Because the entire sample including the skin portion was included in the MPN assay, the lower limit of detection was one cell of *Salmonella* per skin portion or 0 log. MPN tubes were incubated for 24 h at 38°C, and the presence of *Salmonella* in MPN tubes was determined by drop plating 5 µl from each MPN tube onto XLH-CATS for Typhimurium, XLH-NATS for Kentucky, and XLH-TUGS for Hadar. Positive tubes resulted in growth of typical black colonies of *Salmonella* on drop plates after 24 h of incubation at 38°C.

Viable counts. Pulsifate was serially diluted in buffered peptone water, and 50 µl of appropriate serial dilutions were spiral plated (WASP, Microbiology International) onto XLH-CATS for Typhimurium, XLH-NATS for Kentucky, and XLH-TUGS for Hadar. Spiral plates were incubated for 24 h at 38°C before automated counting of black colonies (ProtoCol, Microbiology International). Viable counts were determined when *Salmonella* cells were >3 log per skin portion.

Data processing. MPN were calculated by the method of Thomas (39) and with CFU expressed as logarithmic numbers. When *Salmonella* organisms were not detected in a sample, a value of -0.01 log was assigned because this is the value used for negative samples in ComBase (<http://wyndmoor.arserrc.gov/com-base/>), which is the microbial modeling database in which data from this study will be archived.

Model development. A data set was created in a computer spreadsheet (Excel 2003, MicroSoft Corporation, Redmond, WA) with separate columns for serotype (independent categorical variable), temperature (independent numerical variable), time (independent numerical variable), and logarithmic number (dependent variable). A GRNN model was trained by the method of Specht (35) using a spreadsheet add-in program (version 1.0, Neural Tools, Palisade Corp., Newfield, NY). Eighty percent of the data were used for training and 20% were used for testing.

Model equations. Equations used in the GRNN model were as follows (15):

$$D(S, T, t) = \sum_{j=1}^p \left(\frac{x_j - x_{ij}}{\sigma_j} \right)^2 \quad (1)$$

$$\hat{y}(x) = \frac{\sum_{i=1}^n y_i \exp(-D(S, T, t))}{\sum_{i=1}^n \exp(-D(S, T, t))} = \frac{N(x)}{D(x)} \quad (2)$$

$$e = (\hat{y} - y)^2 \quad (3)$$

where $D(S, T, t)$ is the distance function for independent variables (x) of serotype (S), temperature (T), and time (t), j is the index for independent variables that ran from 1 to p , i is the index for observed values that ran from 1 to n , σ is the smoothing factor, \hat{y} is the predicted value (logarithmic number), y is the observed value (logarithmic number), and e is the squared error.

Model structure. The GRNN model had an input layer with one node per independent variable, a pattern layer with one node per observed value, a summation layer with numerator and denominator nodes, and an output layer (Fig. 1). Each node in the pattern layer computed its distance from the presented observed value (equation 1) and then passed the values to the summation

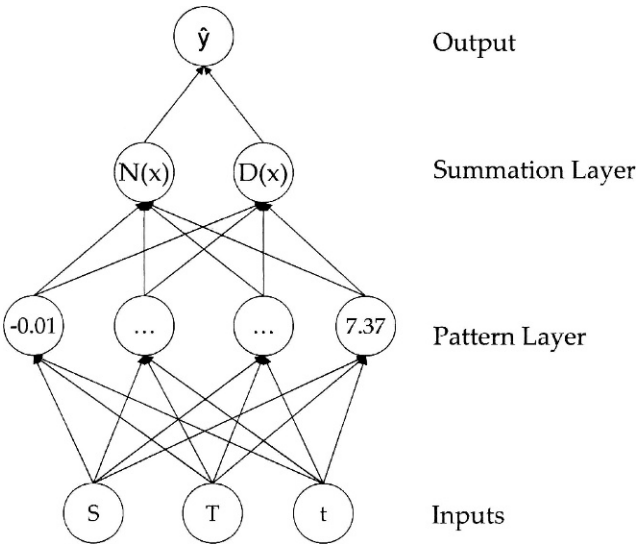


FIGURE 1. General regression neural network for modeling the survival and growth of Salmonella on raw chicken skin as a function of serotype (S), temperature (T), and time (t).

layer (equation 2). The summed values from the numerator node were then divided by the summed values from the denominator node to obtain the predicted value in the output layer. Training of the GRNN involved optimizing smoothing factors to minimize the mean squared error (equation 3).

Model performance. Observed values and predicted values were used to calculate residuals (r):

$$r_i = y_i - \hat{y}_i \tag{4}$$

where r_i (log) is the i th residual, y_i is the i th observed value (log), and \hat{y}_i is the i th predicted value (log). The percentage of residuals in an acceptable zone from -1 log (fail-safe) to 0.5 log (fail-dangerous) was calculated and used as a measure of model performance. Validation occurred when acceptable prediction rates were $\geq 70\%$ (23, 24).

Model simulation. A spreadsheet add-in program (version 5.0, @Risk, Palisade Corp.) was used to simulate the GRNN model. A discrete distribution was used to model serotype prevalence (equation 5), whereas pert distributions (equation 6) were used to model uncertainty of temperatures and times of abuse:

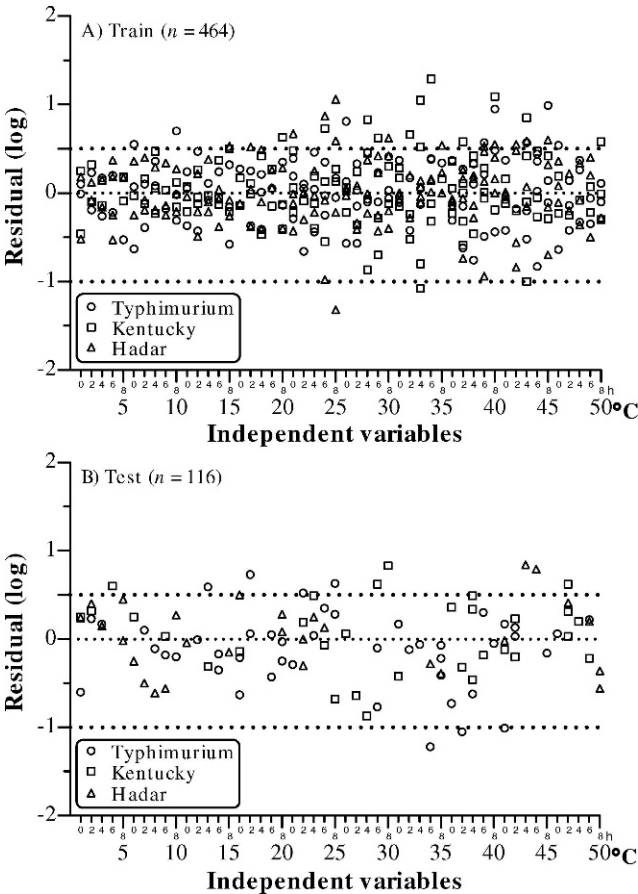


FIGURE 2. Residual plots for (A) dependent data for training and (B) independent data for testing model performance. Residuals were sorted by temperature and then time in ascending order. Major ticks correspond to temperature and 8 h of incubation, whereas minor ticks to the left of major ticks correspond to incubation times of 0, 2, 4, and 6 h, respectively, for the temperature indicated on the major tick. Lower and upper dashed lines are boundaries of the acceptable prediction zone.

$$= \text{RiskDiscrete}[(x_1, \dots, x_i), (p_1, \dots, p_i)] \tag{5}$$

$$= \text{RiskPert}(\text{minimum, most likely, maximum}) \tag{6}$$

where x_i is the value of the i th serotype and p_i is the probability of occurrence of the i th serotype. The model output was the logarithmic change (Δ):

TABLE 1. Input settings and output results for short-term temperature abuse scenarios for demonstrating how the general regression neural network and Monte Carlo simulation model for survival and growth of Salmonella on raw chicken skin as a function of serotype, temperature, and time can be integrated with risk assessment^a

Scenario	Input settings			Output results		
	Serotype (%)	Temp (°C)	Time (h)	Logarithmic change		
	T/K/H	Min/ML/Max	Min/ML/Max	Correlation	Min/50%/Max	Distribution
A	31/58/11	5/20/50	0/2/8	0	−0.21/0.09/4.8	Pearson5
B	31/58/11	5/20/50	0/2/8	−1	−0.16/0.04/0.5	Logistic
C	58/11/31	5/20/50	0/2/8	0	−0.33/0.08/4.2	Pearson5
D	58/11/31	5/20/50	0/2/8	−1	−0.16/0.03/0.5	Pearson5
E	11/31/58	5/20/50	0/2/8	0	−0.29/0.11/4.2	Pearson5
F	11/31/58	5/20/50	0/2/8	−1	−0.33/0.06/0.5	LogLogistic

^a T, Typhimurium; K, Kentucky; H, Hadar; Min, minimum; ML, most likely; Max, maximum; 50%, median.

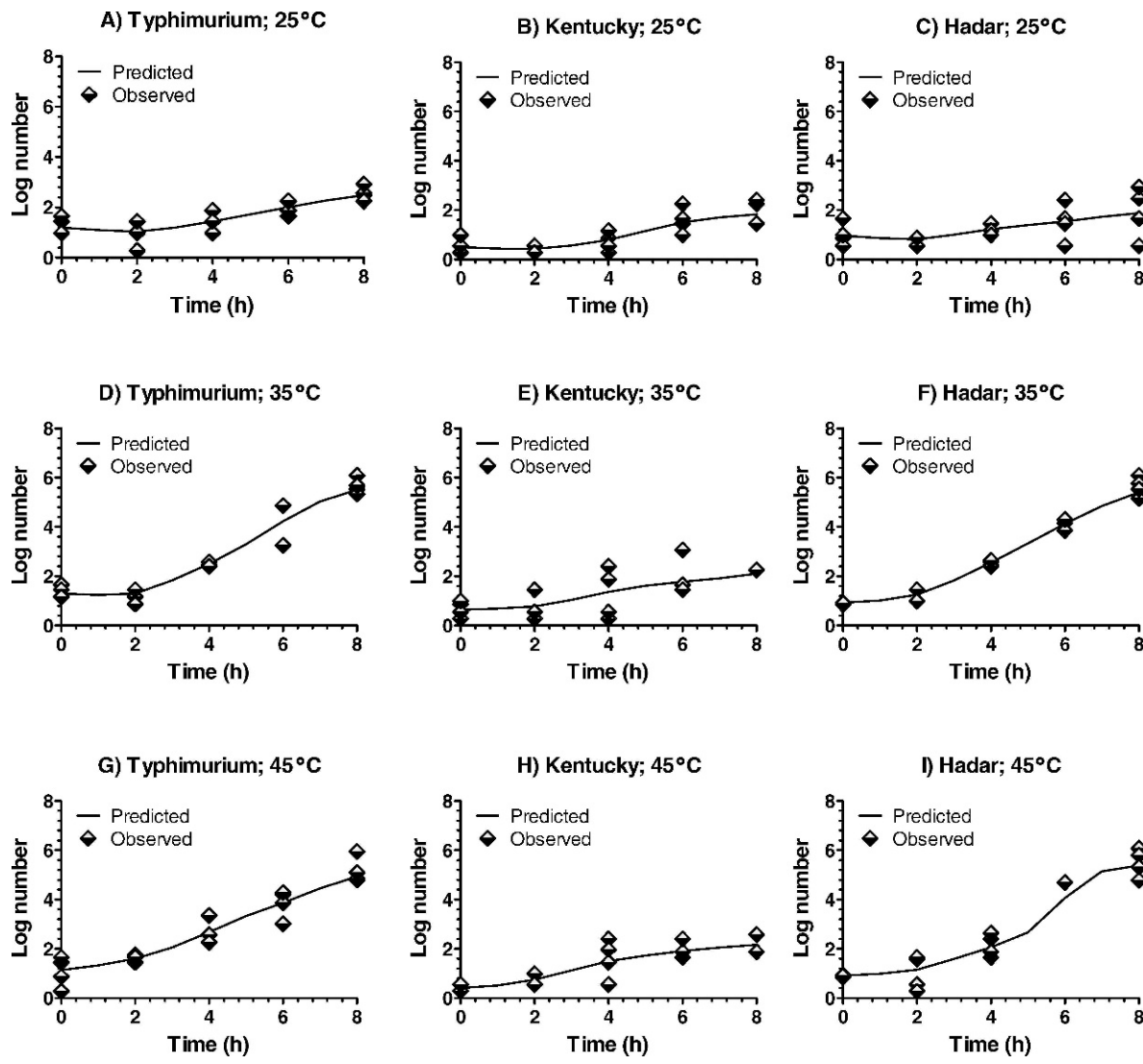


FIGURE 3. General regression neural network model predictions (lines) and observed data (symbols) for logarithmic number of *Salmonella* serotypes Typhimurium, Kentucky, and Hadar on raw chicken skin incubated at 25, 35, or 45 °C for 0 to 8 h.

$$\Delta = \hat{y}(t)_i - \hat{y}(0)_i \tag{7}$$
where $\hat{y}(t)_i$ is the predicted logarithmic number at time t for the i th iteration and $\hat{y}(0)_i$ is the predicted logarithmic number at zero hour for the i th iteration.

Six temperature abuse scenarios with the input settings indicated in Table 1 were simulated for demonstration purposes. Simulation settings were one simulation, Latin Hypercube sampling, Mersenne Twister generator, 10^3 iterations, a fixed random number generator seed of 1, and a correlation between temperature and time of 0 or -1 . The best-fitting distributions for output data (i.e., Δ) were determined using the chi-square statistic within the BestFit function of @Risk.

RESULTS

The GRNN model was trained on 464 data points and had an acceptable prediction rate of 91.8%. There were no signs of systematic prediction bias as a function of serotype, temperature, or time (Fig. 2A). The relative contribution of independent variables was 16.8% for serotype, 48.3% for temperature, and 34.9% for time.

When tested against independent data ($n = 116$; Fig. 2B), the GRNN model had an acceptable prediction rate of 88.8% and did not exhibit systematic prediction bias as a function of independent variables. Thus, the model was validated because its acceptable prediction rates for dependent and independent data exceeded 70%.

Figure 3 shows selected examples of the GRNN model predictions and observed data. Overall, Kentucky exhibited less growth than Typhimurium and Hadar, which had similar growth levels on raw chicken skin with native flora.

The GRNN model predicted logarithmic numbers of *Salmonella* for temperatures and times that were and were not investigated but that were within ranges of independent variables used in model development. For example, growth of Kentucky at 37 °C for 5.3 h, a combination of temperature and time that was not investigated, was 1.35 log (Fig. 4). Output graphs of the GRNN model are shown in Figure 5 and further demonstrate that growth of Kentucky was less than growth of Typhimurium or Hadar, which displayed similar growth levels on raw chicken skin.

The temperature abuse scenarios shown in Table 1 were simulated to demonstrate how the GRNN model can be integrated with risk assessment and how correlation of independent variables can influence and perhaps improve model predictions. In scenarios A, C, and E, temperature and time were not correlated, whereas in scenarios B, D, and F, temperature and time were negatively correlated to simulate the assumption that longer times of temperature abuse were more likely to occur at low temperatures than at higher temperatures.

Use of a -1 correlation between temperature and time produced a large decrease in the upper tail of the output distributions with little or no effect on the lower tail or median of the output distributions (Table 1 and Fig. 6). In the absence of correlation, the maximum logarithmic changes were 4.8, 4.2, and 4.2 for scenarios A, C, and E, respectively, and output data fit best to a Pearson5 distribution. In the presence of correlation, the maximum logarithmic change was 0.5 for scenarios B, D, and E, and output data fit best to a Logistic, Pearson5, and LogLogistic distributions, respectively. These data illustrate that correlation of independent variables is a modeling technique that can be used to improve model performance by avoiding unlikely temperature abuse scenarios (e.g., 6.9 h at 40°C) that might result in overly fail-safe predictions of *Salmonella* growth.

Table 2 shows the combinations of independent variables in scenarios A and B that resulted in the top five largest increases in the logarithmic number of *Salmonella* on raw chicken skin subjected to temperature abuse. These results demonstrated that the upper tail of the output distribution for scenario A resulted from extended temperature abuse at near-optimal or optimal growth temperatures for *Salmonella*; such scenarios are hopefully not likely to occur in the real world. In addition, these results demonstrated that a correlation of -1 might have been too strong of a correlation between temperature and time of abuse, as the top five iterations in scenario B were all for a low temperature and indicate that some realistic scenarios, such as 22°C for 4 h, were excluded and thus resulted in overly fail-dangerous predictions of *Salmonella* growth during temperature abuse.

The correlation value between 0 and -1 that reduces or eliminates predictions that are overly fail-safe or overly fail-dangerous was not determined and is likely to differ as a function of the settings for the input distributions that define the times and temperatures of abuse. Rather, the scenarios simulated were designed to demonstrate the extremes of the correlation effect and the importance of this aspect of the model on output results for use in risk assessment.

DISCUSSION

Research in predictive microbiology most often involves development of predictive models in pure broth culture with a high initial dose of test pathogen. The operating hypothesis is that models developed in pure broth culture as a function of the major factors (i.e., temperature, pH, and water activity) controlling pathogen growth provide

reliable predictions for food. Valid reasons for this approach are technical ease, reduced cost, and potential model robustness. Some historical examples of this approach are the works of Gibson et al. (10), Buchanan and Phillips (2), and Sutherland et al. (37).

Accurate and unbiased predictions of pathogen growth are needed to safeguard public health. Models that underpredict pathogen growth result in consumption of unsafe food, whereas models that overpredict pathogen growth result in destruction of safe food, which is not desirable. By not considering microbial competition, models developed in pure broth culture overpredict pathogen growth. For example, maximum growth of *Salmonella* in pure broth culture at 10°C is 9 log/ml (10), whereas maximum growth of *Salmonella* on ground chicken breast meat with native flora is 2 log/g (26), a difference of 7 log.

The availability of pathogen isolates with a phenotype that can be detected in the presence of other microorganisms is a hurdle for development of predictive models in food with native flora. Transformation of pathogens by mutation or molecular cloning to a phenotype (e.g., antibiotic resistant, fluorescent, or luminescent) that can be detected in food with native flora often results in fitness problems that invalidate the use of the new strains for development of predictive models (32). For example, *Salmonella* cells transformed to express the green fluorescent protein from a jellyfish are visible for detection and enumeration in predictive microbiology studies but grow more slowly than parent strains and thus are not good strains for model development (19, 29). In contrast, *Salmonella* isolates with natural resistance to antibiotics can be used to model growth on chicken products with native flora by using plating media with antibiotics that suppress and eliminate native flora during enumeration (25–27). The latter approach was used successfully in the present study to develop a predictive model for survival and growth of three serotypes of *Salmonella* on raw chicken skin with native flora.

Most studies in predictive microbiology use a mixture of pathogen strains for model development. The idea is that this will result in a fail-safe model because the fastest-growing strain will predominate under the conditions tested. However, models developed with a cocktail of strains could be overly fail-safe. For example, if the present model had been developed with a cocktail of Typhimurium, Kentucky, and Hadar, the faster-growing serotypes Typhimurium and Hadar would have predominated and the resulting model would have overpredicted growth of *Salmonella* on raw chicken skin contaminated with the slower-growing serotype Kentucky. For example, in Figure 5A, the GRNN model would have overpredicted growth of Kentucky by ca. 3 log at 8 h of temperature abuse at 37°C. Thus, by developing models with individual strains and then modeling growth as a function of serotype prevalence, more accurate predictions are obtained.

From an epidemiological perspective, *Salmonella* serotypes are classified as host adapted, host restricted (HR), and unrestricted (40). Serotype Gallinarum is HR for poultry, whereas Typhimurium is unrestricted. Auxotrophy is characteristic of HR serotypes (40), and this characteristic

	A	B	C	D	E	F	G	H
1	Input	Output	Typhimurium	Kentucky	Hadar			
2	Serotype (%)	Kentucky	31	58	11			
3			Minimum	Most Likely	Maximum	Correlations	Temp	Time
4	Temp (5 to 50C)	37.0	5	20	50	Temp	1	
5	Time (0 to 8 h)	5.3	0	2	8	Time	0	1
6								
7	Serotype	Temp	Time	Log				
8	Kentucky	37.0	5.3	1.97				
6	Kentucky	37.0	0	0.62				
10			Log change	1.35				

FIGURE 4. Spreadsheet layout of the general regression neural network and Monte Carlo simulation model for predicting logarithmic change (cell D10) of *Salmonella* on raw chicken skin as a function of serotype prevalence (cells C2:E2) and uncertainty of temperatures (cells C4:E4) and times (cells C5:E5) of abuse. For the iteration shown, the outputs of the input distributions were serotype Kentucky (cells B2, A8, A9), 37°C for the temperature of abuse (cells B4, B8, and B9), and 5.3 h for the time of abuse (cells B5 and C8), whereas the outputs of the model were a logarithmic number of 1.97 after 5.3 h at 37°C (cell D8), an initial logarithmic number of 0.62 (cell D9), and a logarithmic number change of 1.35 after 5.3 h at 37°C (cell D10). The correlation between time and temperature of abuse was 0 (cell G5) for the scenario simulated.

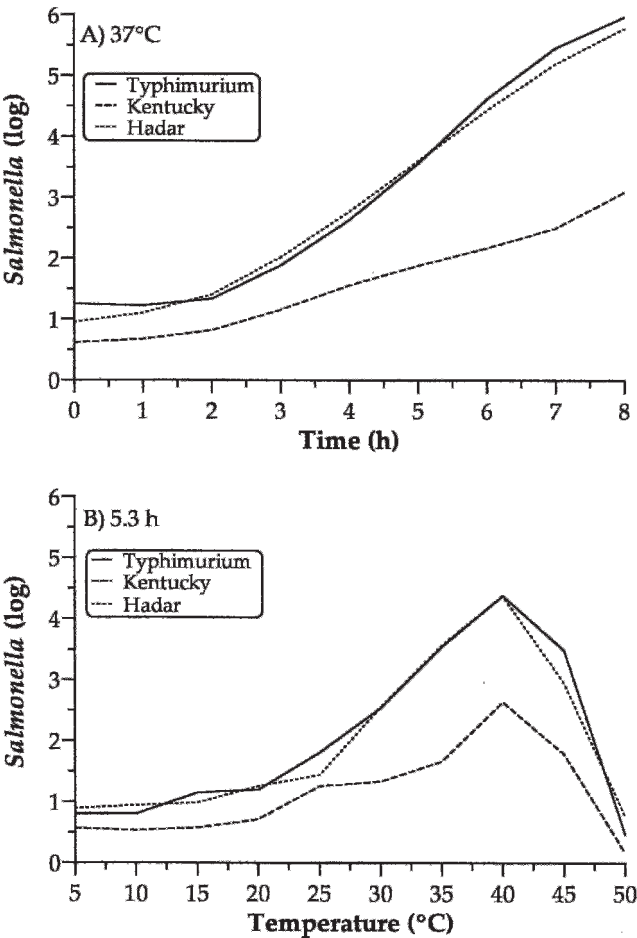


FIGURE 5. Output graphs from the general regression neural network model for survival and growth of *Salmonella* on raw chicken skin as a function of (A) time at 37°C and (B) temperature at 5.3 h for the scenario in Figure 4.

could limit growth of HR serotypes on food with native flora where certain nutrients might be in short supply. It is interesting to speculate that Kentucky might be HR for meat-type chickens, as this serotype is not a top 20 serotype in humans, swine, or turkeys (6), and this could possibly explain its poor growth on chicken skin in the present study.

Competitive exclusion of one serotype of *Salmonella* by another serotype has been hypothesized to explain the emergence of the Enteritidis epidemic in chicken eggs following elimination of Gallinarum by depopulation of infected layer-type chickens (14). If Kentucky proves to be an HR serotype for meat-type chickens, it might be desirable to not reduce or eliminate this serotype so that it could competitively exclude other highly virulent serotypes (e.g., Typhimurium) in humans from colonizing chickens. The lower growth of Kentucky on raw chicken skin in this study indicates that it would pose much less risk than Typhimurium and Hadar on chicken that has been subjected to short-term temperature abuse. However, more information on the pathogenic potential of Kentucky in humans and meat-type chickens is needed before such a strategy is further contemplated.

Models in predictive microbiology are usually developed in three stages using regression methods. First, kinetic data for growth are fit to a primary model that predicts changes in pathogen number as a function of time. Second, growth parameters (e.g., lag time and growth rate) from primary modeling are fit to secondary models that predict them as a function of independent variables. Third, primary and secondary models are combined to form a tertiary model that predicts pathogen growth as a function of time and independent variables. Limitations of this approach are that it is time-consuming, requires significant training in regression analysis, and uses regression models that are inflexible (i.e., have a defined response surface of prediction).

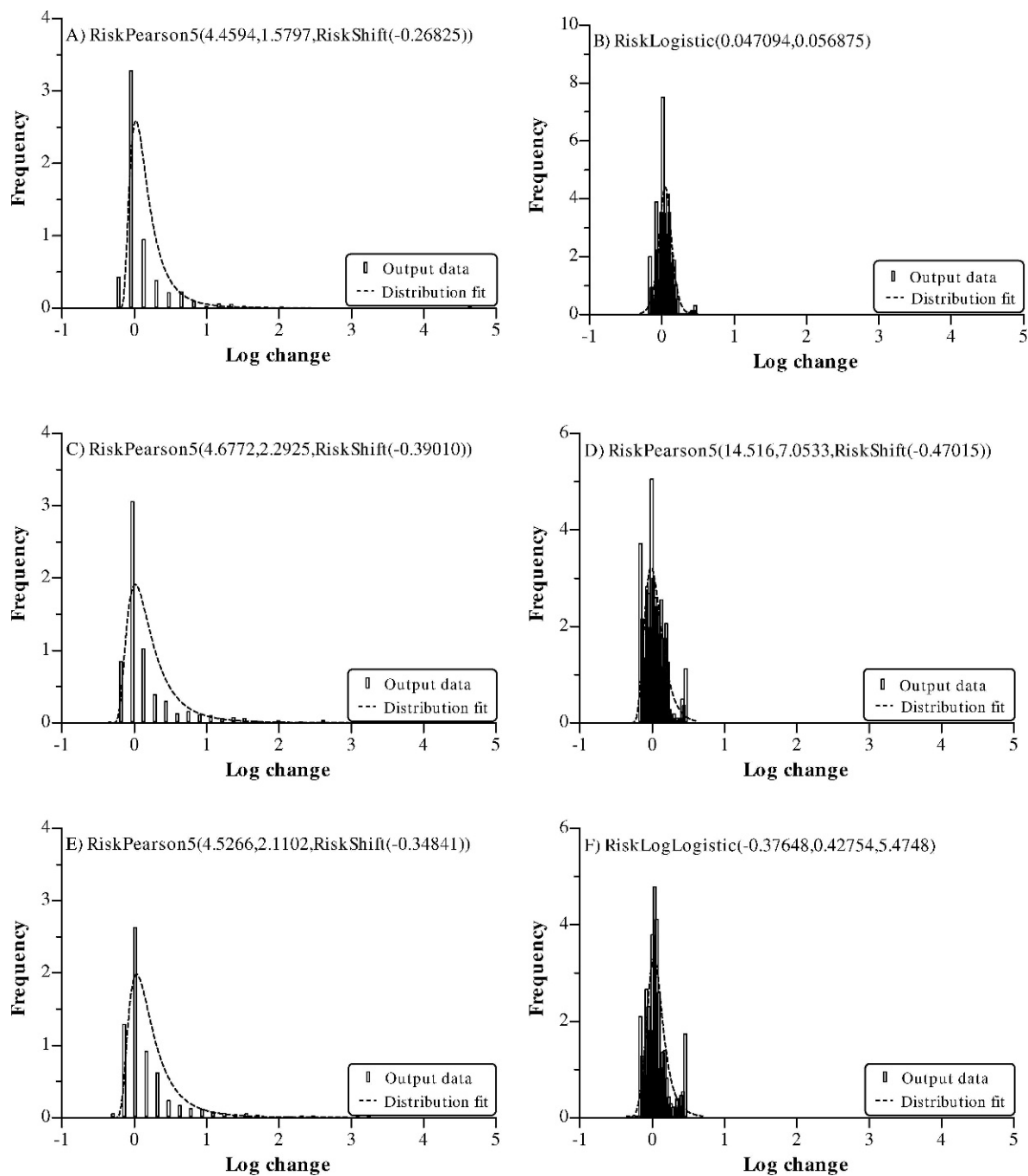


FIGURE 6. Output data and best-fit distributions from the general regression neural network and Monte Carlo simulation model for the scenarios listed in Table 1.

Neural network modeling overcomes limitations of regression modeling as it is fast, requires only a basic understanding of the method, is flexible, and outperforms regression modeling in predictive microbiology applications (4, 7–9, 11–13, 30). These studies all used regression for primary modeling and neural networks for secondary modeling. In the present study, a general regression neural network was used in one step for primary, secondary, and tertiary modeling, and the resulting model had acceptable and high performance (ca. 90% acceptable predictions). Thus, it does not seem necessary to use regression modeling in tandem with neural network modeling when neural network modeling is capable of developing predictive

models in one step with considerable savings in time, effort, and performance.

Risk assessment provides stochastic predictions of the risk of adverse health outcomes from food produced by different farm-to-table scenarios. Predictive models are used in risk assessment to provide stochastic predictions for individual pathogen events, such as initial contamination (22), growth (18), thermal inactivation (20), and dose-response (21). Consequently, the GRNN model developed in this study was configured for risk assessment by using Monte Carlo simulation in tandem with GRNN modeling software to provide stochastic predictions of relative growth (i.e., logarithmic change) of *Salmonella* without specifying a

TABLE 2. Top five iterations for logarithmic change in scenarios A and B in Table 1 for survival and growth of *Salmonella* on raw chicken skin exposed to short-term temperature abuse

Scenario/ iteration	Logarithmic change	Serotype	Temp (°C)	Time (h)
A/457	4.81	Hadar	40.0	6.9
A/555	2.20	Typhimurium	35.0	5.2
A/991	2.13	Kentucky	37.7	6.4
A/496	2.11	Typhimurium	35.9	5.1
A/330	1.95	Typhimurium	32.6	5.3
B/516	0.49	Hadar	14.7	4.0
B/875	0.49	Hadar	14.5	4.0
B/462	0.49	Hadar	14.5	4.0
B/568	0.49	Hadar	14.8	3.9
B/505	0.49	Hadar	14.4	4.0

denominator (e.g., per square centimeter). By not having a denominator, relative growth predictions can be seamlessly integrated with risk assessments that express results in different forms, such as per gram, per square centimeter, per serving, or per carcass.

The rationale for no denominator was based on a consideration of what is known about the ecology of *Salmonella* on chicken. Surkiewicz et al. (36) reported that a 2.5-lb chicken carcass has a surface area of 1,900 cm² and that the distribution of the number of *Salmonella* among chicken carcasses is not uniform but rather is as follows: 79% contain no *Salmonella*, 16% contain 1 to 30 cells, 1% contain 30 to 300 cells, and 4% contain >300 cells. Thus, expression of results per square centimeter would have been based on a false assumption, i.e., uniform distribution of *Salmonella*. Rather than make such a false assumption, it was assumed that *Salmonella* was distributed in clusters and that growth occurred in colonies.

The most widely used method for evaluating model performance in predictive microbiology is the bias (B_f) and accuracy (A_f) factor method of Ross (33). This method normalizes predicted values to homogenize variance and was originally developed to evaluate the performance of secondary models for generation time. An A_f of 1.1 indicates that on average there is a 10% difference between observed and predicted values.

Tamplin et al. (38) used B_f and A_f to evaluate performance of secondary models for growth parameters. The authors reported A_f values of 1.14 for specific growth rate (measured in log per hour), 1.33 for lag-phase duration (measured in hours), and 1.02 for maximum population density (MPD; measured in log per gram) for *Escherichia coli* O157:H7 and ground beef incubated at 5 to 46°C. These results indicate that on average observed and predicted values differ by 14, 33, and 2% for specific growth rate, lag-phase duration, and MPD, respectively.

A 10% difference in MPD or an A_f of 1.1 is obtained for a predicted value of 10 log and an observed value of 9 log. The residual for the latter prediction case is 1 log, which corresponds to a 90% difference in pathogen numbers. By using log-transformed values for MPD to calculate A_f , Tamplin et al. (38) overestimated model performance

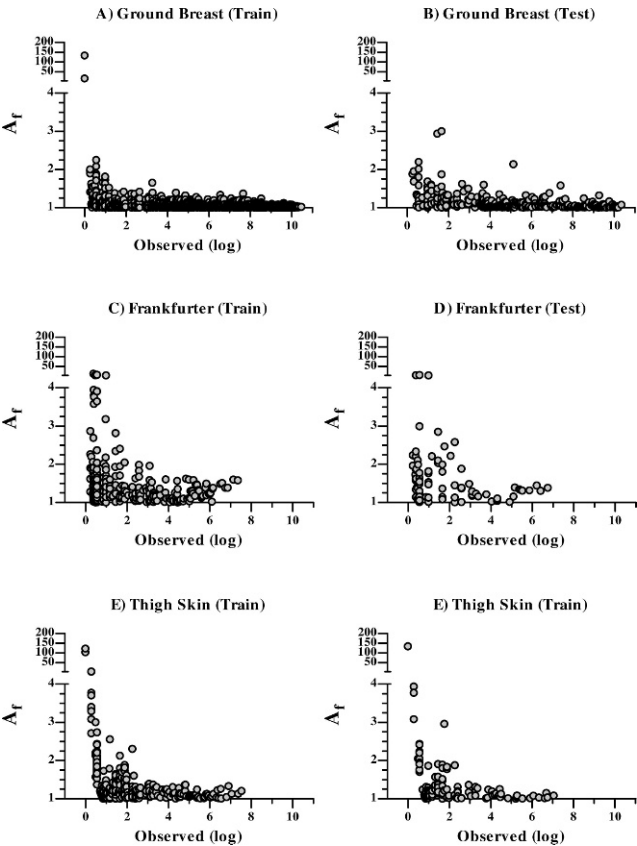


FIGURE 7. Accuracy factor (A_f) plots for performance of general regression neural network models developed using data for raw ground chicken breast meat (25), chicken frankfurters (27), and raw chicken skin (28). The performance evaluation with A_f was conducted with dependent data used to train the model and independent data used to test the model.

because they did not consider that logarithmic transformation is also a method used to homogenize variance in predictive microbiology.

When observed logarithmic values approach 0 log, methods that normalize predicted values (38) or that normalize residuals (33) result in large values that inflate mean bias and accuracy factors (3). For example, an A_f of 98 is obtained for a predicted value of 0.98 log and an observed value of 0.01 log. To further illustrate this point, log MPN and log CFU data from previous modeling studies for *Salmonella* growth from a low initial dose (<1 log) on chicken products with native flora (25, 27, 28) were used to train and test GRNN models, and then performance of the models was evaluated by graphing A_f values for individual prediction cases as a function of observed values (Fig. 7). These results illustrate that A_f values are large when observed values are small and A_f values are small when observed values are high. Thus, for models that predict logarithmic number, such as the GRNN model in this study and the MPD model of Tamplin et al. (38), it is best not to normalize predicted values or residuals when evaluating model performance, as this results in an inaccurate assessment of model performance.

Jeyamkondan et al. (13) compared statistical indices of model performance and concluded that model performance

is better evaluated graphically and that an objective measure of model performance is needed only when error plots are similar. The acceptable prediction zone method used in this study is a graphical method that uses a single quantitative factor (i.e., acceptable prediction rate) to evaluate model bias and accuracy in one step. A concept used in the acceptable prediction zone method is that model predictions can deviate from observed values more in the fail-safe than fail-dangerous direction when a model is used to predict food safety (34). A second concept used in the acceptable prediction zone method is that models can exhibit prediction bias as long as that prediction bias is at an acceptable level in either the fail-safe or fail-dangerous direction.

The acceptable prediction zone method has been shown to provide a more complete and accurate assessment of model performance than B_f and A_f (24). In the present study, the GRNN model had an acceptable prediction rate of 92% for dependent data and 89% for independent data. Since these values exceeded 70%, which is the criterion for acceptable model performance, the GRNN model was classified as validated for predicting survival and growth of *Salmonella* from a low initial dose on raw chicken skin as a function of serotype, temperature, and time. Thus, the model can be used with confidence in hazard analysis and critical control point and risk assessment. However, because parameters of the GRNN model are not provided by the Neural Tools software application, deployment of the model to stakeholders might be limited by the requirement that users possess the Neural Tools and @Risk software used to run and simulate the model and make predictions. The robustness (i.e., ability to extrapolate) of the model for predicting growth and survival of *Salmonella* from higher initial doses and on other poultry meats and poultry meat products is currently being investigated.

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